

Effects of gut microbiome and environment on the development of eczema in Chinese infants

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Abstract

Background: Eczema is a relapsing and persistent inflammatory skin disease affecting about one-fifth of children worldwide. As in other developed countries, the prevalence of this chronic disease in Hong Kong is approximately 30%. Moreover, the number of local cases reported has been on a rising trend since 1995. Eczema frequently starts in early infancy. A total of 45% of all cases begin within the first six months of life, 60% during the first year and 85% before the age of 5. The pathophysiology of eczema is multi-factorial and is a complex inter-relationship between skin barrier, genetic predisposition, immunologic development, microbiome, environment, nutrition, and pharmacological and psychological factors.

Objective: To characterize the longitudinal changes of gut microbial profile in early childhood and to examine the association between gut microbiome diversity, environmental factors and the development of eczema in early childhood.

Method: We will conduct a longitudinal cohort study that follows 1250 Hong Kong Chinese infants for 2 years and assess the gut microbiome and other potential environmental factors in the aetiology of eczema. Parents will be asked to provide demographic data, their infant birth data, allergy condition, diet, environmental conditions as well as the data on maternal stress. Stool specimen will be collected for gut microbiome diversity analysis. We will examine newborn infants at enrollment, at 4 months, 1 year and 2 years after birth.

Expected results: This study will evaluate the association between gut microbiome, environmental factors and the development of eczema in Chinese infants. Findings from this study may be used to develop a predictive path model to guide effective health promotion, disease prevention and management.

Abbreviation: SCORAD = scoring atopic dermatitis.

Keywords: eczema, environmental factors, maternal stress, microbiome

1. Introduction

Eczema is a chronic and relapsing inflammatory skin disease affecting about 20% of children and 3% of adults worldwide.^[1,2] Approximately 70% of cases of eczema start in early childhood, under 5, and only about 10% in adulthood.^[3] Among the cases of early childhood onset, about 50% develop symptoms within the

first year of life.^[1] In Hong Kong, the prevalence of eczema is on a rising trend, a 0.12% increase per year since 1995.^[4] Summarizing the results of the International Study of Asthma and Allergies in Childhood, Chan et al^[4] noted that the prevalence of life-time eczema in children was 30.7%.

The persistence of eczema incurs substantial healthcare costs and impairs the social development of patients because of a poor self-image and lack of self-confidence.^[5] It also imposes financial and psychological burdens on the family. Complications of bacterial superinfection and bacteraemia can also be lethal in children.^[6] Eczema is always the first step in the atopic march and up to 80% of patients will develop asthma, allergic rhinitis or hay fever later in their lives.^[2,7] It is necessary to identify and understand factors in the development of the disease in early childhood which may be pivotal to the onset of the atopic march.

Two theories concerning the component of skin inflammation have been proposed.^[8] One holds that the essential deformity dwells in an immunologic aggravation that causes IgE-intervened allergy, with epithelial-boundary brokenness viewed as a result of the neighborhood irritation. The immune reaction may be influenced and driven by environmental stimuli such as allergens resulting from poor air quality or from a diet which may change the gut microbial components. Another speculation recommends that an inherent deformity in the epithelial cells prompts the hindrance brokenness, specifically the microbial substances on the skin. The immunological aspects are considered to be an

This study was supported by the Department Funding from The Nethersole School of Nursing in The Chinese University of Hong Kong.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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How to cite this article: Chan CW, Leung TF, Choi KC, Tsui SK, Chan JY. Effects of gut microbiome and environment on the development of eczema in Chinese infants. *Medicine* 2020;99:21(e20327).

Received: 17 April 2020 / Accepted: 20 April 2020

<http://dx.doi.org/10.1097/MD.00000000000020327>

epiphenomenon.^[8] Irritation, lack of sleep and social humiliation due to visible lesions have substantial effects on the psychosocial prosperity of patients and their family members.

The microbial composition is involved in the development of the regulatory T-cell response and thereby plays a key role in immune development in the case of eczema.^[9] Inside *in vitro* measures, it has been indicated that the expansion of explicit oligosaccharides during dendritic cell advancement initiates an administrative T-cell reaction of potential advantage in an unfavorably susceptible setting.^[10] Moreover, dietary supplementation with specific prebiotic oligosaccharides has been shown to reduce the risk of developing allergies in infants.^[11] During early life it therefore seems likely that specific components of the microbial composition can contribute to the normal immune development via multiple direct and indirect pathways, thereby reducing the risk of allergic diseases. Other recently reported birth cohort studies have addressed the relationship of the intestinal microbiome and allergic outcomes, with most suggesting associations.^[12]

The main contact of mucosal tissues with outside microbiota is vital in the foundation and development of the mucosal, as well as systemic immune systems.^[13] In particular, the first year of life is essential to programming the immune system. The development of a barrier function and the immune system are influenced by environmental factors, such as feeding patterns, antibiotic use by the mother during delivery and the use of antibiotics by the neonate.^[14] Proper understanding of the protective and programming effects of healthy immune and microbiome development may provide opportunities to reduce the risk of developing eczema. Culture-free studies have given more prominent knowledge into the worldly variances in the bacterial network assorted variety as it creates during a newborn child's first year of life, including dramatic decreases in diversity on antimicrobial administration.^[15] However, by approximately 12 months of age, this gut community begins to resemble that of an adult-like microbiome, dominated by the bacterial phyla Firmicutes and Bacteroides and including members of the Proteobacteria, Actinobacteria and Verrucomicrobia, among others.^[15] Thus, it has been suggested that this underlying time of colonisation, which coincides with immune response development, represents a vital window during which deviations in colonisation patterns may influence normal immune maturation.^[16] Investigators can now take advantage of advances in 16S rRNA gene sequencing and deep sequencing technologies that allow comprehensive detection of microbial communities on a large scale.^[17]

The prevalence of eczema appears to vary worldwide, with rates ranging from 1% to 20% among children and adults,^[5] possibly due to the role of environmental factors in the expression of the disease. Although epidermal gene mutations will have an effect on skin barrier function and provoke an inclination towards immune deviation within the direction of allergic reaction, not all patients with eczema have these mutations. It has been suggested that certain environmental stimuli may be able to trigger both epidermal damage and a Th2 immune reaction regardless of genetic background.^[18] In an article published in *Science* in 2016,^[19] it mentioned that "Because microbe-immune interactions appear to play an important role in early-life development of the immune system and when disrupted may result in potentially persistent immune abnormalities, it is worth considering whether early-life exposure to microbes may affect later-life susceptibility to disease. There is emerging evidence to support this hypothesis . . .". However, the data

on early-life environmental factors and microbiota composition in Hong Kong infants is insufficient. Other environmental factors also play a part. A recent integrative review reports that infant gestational age, delivery mode and feeding type were found to affect gut microbiome composition.^[20] Exposure to some antigens seems to increase tolerance and lower the risk of eczema in infants.^[21] Dog ownership, the presence of older siblings and probiotic treatment are factors associated with reduced likelihood of allergies. Socio-economic factors, including educational level and parental stress, are also associated with the prevalence of the disease. However, the question of a mechanism linking environmental influences, microbial composition and eczema remains inconclusive,^[22] and further scientific investigation is required to confirm or reject.

Both gut microbiome and environmental factors are essentially concerned in the development of eczema, and are also associated with the severity of the disease. In the light of this, we propose to initiate a birth cohort study, examining newborn infants at enrollment, 4 months, 1 year and 2 years after birth. We will access their gut microbiota profile, and measure different environmental factors that may be associated with the disease. This cohort study will help us to understand and pinpoint the gut microbiome and different environmental factors involved in the development and severity of eczema in Chinese children.

2. Methods

2.1. Study design

This will be a prospective birth cohort study of 1250 Chinese newborns. The subjects will be recruited from Department of Paediatrics in Prince of Wales Hospital in Hong Kong. Subjects' parents will be asked to provide demographic data, their infant birth data and allergy condition. The cohort will be followed for 2 years, with 3 follow-up measurement points to assess the diet, environment and allergy condition of the infants at the ages of 4 months, 1 year and 2 years. Parental stress will also be assessed. Stool specimens from the newborns will be collected for gut microbiome diversity testing at enrollment, 4 months, 1 year and 2 years of age. It is expected that the whole study will take 36 months to complete.

2.2. Subject recruitment

1250 new-born infants will be recruited and followed for two years in the Department of Paediatrics of the Prince of Wales Hospital in Hong Kong. We will recruit full-term infants from the postnatal ward. General population cohort will be included with regardless of family history. The inclusion criteria are in Chinese ethnicity and reside in Hong Kong. The infants with GI disorders after birth, received intravenous injection of antibiotics, admitted to neonatal ICU, whose mothers have fever or an infection or are currently taking antibiotics will be excluded.

2.3. Ethical considerations

This study was registered on the ISRCTN Registry with reference number ISRCTN44080054. The study protocol was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee with reference number 2016.321. Any modifications to the protocol that may have an impact on the execution of the study will be reported to

the committee. Potential subjects' parents will be given detailed information about the study and their rights to participate. Written informed consent will be obtained from the subjects' parents prior to data collection.

2.4. Sample size estimation

1250 new-born infants will be recruited. The hospital averages of 5000 deliveries per year. According to our anecdotal prevalence data, about 30% (~375 cases) of the infants will develop eczema in the first 2 years of life. It is estimated that an accumulation of 375 event cases would be sufficient to detect a hazard ratio of a standardised normally distributed continuous risk factor and a binary risk factor as small as 1.16 and 1.94 respectively, with 80% power at a 1-sided 2.5% level of significance, on the basis of the very mild assumption that the prevalence of underlying binary risk factors is not too extreme, that is, within 5% to 95%. Moreover, this sample size would also be adequate to detect a binary risk factor with a risk ratio (RR) as small as 1.42 to 2.00 and a normally distributed continuous risk factor with a RR as small as 1.17 to 1.44 at 80% power and 1-sided 2.5% level of significance, provided the prevalence of the underlying binary risk factor was within 5% to 95% and the probability of eczema at the mean value of the continuous risk factor was also within 5% to 95%. This study also aims to examine the mediating role of microbiome diversity on the relationships between environmental and lifestyle risk factors and development of eczema using path analysis. A commonly used rule of thumb^[23] suggests that a sample size of at least 100 and preferable ≥ 200 is required for conducting path analysis. In consideration of both statistical and financial criteria, a random subsample of totally 150 children of the cohort will be undergone gut microbiome examination of their stool specimens collected.

2.5. Data collection

2.5.1. Demographic characteristics. Demographic data will be collected at enrolment by self-report. This information includes: gender, body weight and body length of the infant, gestational age, mode of delivery, feeding practice (breast or formula feeding), emollient use, parents' education level and household income.

2.5.2. Allergy condition. The infant's allergy condition will be assessed by the modified parent proxy version of the Comprehensive Early Childhood Allergy Questionnaire developed by Minasyan and colleagues. Data will be collected by a research assistant at enrolment, and at the age of 4 months, 1 year and 2 years.

Comprehensive Early Childhood Allergy Questionnaire is designed to detect eczema, asthma and IgE-mediated food allergies in children aged from 1 to 5. It assesses allergic diseases, family history of allergy, parental socio-economic status, parental smoking and child's feeding patterns. It has been validated with good psychometric properties and has been translated and modified to reflect the Chinese situation under the standard procedure before being used in this study. Some questions, such as those concerned with the history of infection, use of antibiotic, pet ownership, air quality, number of siblings and mode of delivery, have been added to the questionnaire. The modified questionnaire has been reviewed by an expert panel of paediatricians, geneticists, paediatric nurses, academics, medical scientists and parents of children with eczema. The feasibility,

appropriateness and acceptability of the questionnaire are confirmed by five proxy parents of eczematous children, taking an average of 7 minutes to answer the questionnaire in full.

2.5.3. Diet and use of supplements. The subjects' dietary factors will be assessed by a parent proxy dietary practice questionnaire, adopted from the Chinese version of the eating-habit module of the Behavioural Risk Factor Surveillance System, Hong Kong Centre for Health Protection. The information will be collected from parents when the children are at the age of 4 months, 1 year and 2 years. Information on the mother's diet and use of supplements during pregnancy will be collected after the birth by means of the same questionnaire.

2.5.4. Maternal stress. Maternal stress will be assessed at 4 time points: at enrollment, at 4 months, 1 year and 2 years. Mothers will be asked to complete the 10-item perceived stress scale to assess their own stress levels. There are 10 items, measured on a five-point Likert scale, from 0 (never) to 4 (very often). The Chinese version has been reported to have good validity and internal consistency.^[24]

2.6. Gut microbiome metagenomics analysis

A total of 150 infants who confirmed to have eczema based on the assessment by the Scoring Atopic Dermatitis (SCORAD) index at 2-year will be selected for microbiome diversity profiling. A similar infant microbiome study has demonstrated that a sample size of 100 is enough for the detection of significant changes in the gut community composition.^[25] Infants will be followed for 2 years for development of eczema and other allergic diseases such as recurrent wheeze, food allergy and rhinitis, and the stored stool specimens of a stratified random subsample of 150 infants will be selected for gut microbiome analysis on the basis of severity of eczema as assessed by the SCORAD index at 2-year. Fifteen children in each of the 10 strata based on the deciles of the SCORAD index will be randomly selected using computerized randomization. Stool samples from all newborns will be collected from the napkin and suspended in 0.6 mL lysis buffer (TIANGEN Biotech, Beijing, China). The colonisation and development of the gut microbiome will be followed at 10 days post-birth, 4 months, 1 year and 2 years (modified from Storrø et al^[22]). Stool samples will be stored at -80°C before analysis. Total microbial DNA will be extracted from the stool using the TIANamp stool DNA kit. The quantity and quality of the total microbial DNA will be verified by the NanoDrop 1000 spectrophotometer (Thermal Scientific, Texas, USA) and Bioanalyser 2100 (Agilent Technologies, California, USA) respectively. A total of 600 metagenomic libraries from 4-time points (at enrollment, 4 months, 1 year and 2 years) of 150 infants will be constructed using the PCR primers flanking the V3-V4 region of the 16S ribosomal RNA gene. All PCR will be performed in triplicate to minimise the effect of amplification bias. Following PCR using the high-fidelity enzyme, amplicons with different barcodes will be analysed using the Bioanalyser 2100 and Qubit spectrophotometer. Equimolar amounts of amplicon libraries (~80 libraries per run) will be pooled for next-generation sequencing using the MiSeq platform (Illumina, California, USA).

2.7. Data processing analysis

The metagenomic data set will be analysed using the open source bioinformatics tool Mothur. Briefly, cleaned sequence reads will

be trimmed and aligned to the bacterial-subset SILVA, followed by filtering to remove vertical gaps. The reads are then screened for potential chimeras using the uchime method and finally classified using the Ribosomal Database Project's naïve Bayesian classifier training set for Mothur. Differentially abundant bacterial taxa, PcoA loadings, species richness (Sobs), Shannon's diversity (H') and evenness (EH) and Simpson's diversity will be calculated using the Mothur workflow.

Data will be suitably transformed, if applicable, before being subjected to inferential analyses. In particular, the frequency data of relative abundance of the differentially abundant bacterial taxa identified will be arc-sine square root-transformed. A mixed-effects model will be used to identify environment and lifestyle risk factors associated with the development of eczema by using a log-binomial link function for estimating the relative risks of the factors identified based on the whole cohort. If a more exact time of eczema development can be recorded, Cox regression with possibly time-varying covariates will be used instead to examine environment and lifestyle risk factors associated with the development of eczema. A mixed-effects model will also be used to examine the longitudinal association of gut microbiome diversity in the development of eczema by using the sub-sample with microbiome data. Furthermore, path analysis will be performed to examine whether the mediating role of microbiome diversity in the relationships between environmental risk factors and the development of eczema based on the random sub-sample. The significance of the mediating effects will be determined by bias-corrected bootstrapping method with 10,000 replications. The bootstrapping will be performed using Matlab 7.0 (The Mathworks, Inc). Except as specified above, all other statistical analyses will be performed using SAS release 9.4 (SAS Institute, Cary, NC).

3. Discussion

Eczema is a common chronic inflammatory skin disorder that is regarded as a typical multifactorial disease. It affects up to 230 millions of people globally.^[26] Onset is usually in the first few weeks and months of life and, while most affected people enter spontaneous remission, the dermatitis may persist into adult life. The etiology is complex and the disease is caused by a combination of immune dysregulation, genetic susceptibility, environmental factors and impaired barrier function.^[27,28]

There is a substantial economic cost not only to the family of the person with eczema but also to health services.^[29] In 1 study, a published work review was conducted to estimate the economic burden of pediatric eczema patients in the Asia-Pacific region and showed that the annual direct cost of eczema ranged from USD199 to USD1,250.^[30] Although there is currently no cure for eczema, a wide range of treatments are used to control the symptoms. One such approach is the change of gut microbiome through the diet. Colonization of the infant gastrointestinal tract by microbes is an essential process in our life cycle since microbiota-host interactions have an important influence on human health and disease.^[15] Infancy is a critical period for the colonization of the gut microbiome, which affects host immune development. Following birth, the human intestine is rapidly colonized by an array of microbes and factors known to influence colonization include gestational age, mode of delivery (vaginal birth vs assisted delivery), diet (breast milk vs formula), sanitation, and antibiotic treatment.^[31,32] Therefore, perturbation of the infant gut microbiome can shape the development of

the immune system and link to the risk of allergic disease including atopic dermatitis.^[31,33] People with eczema have different bacteria in their gut compared to people without eczema, and sometimes they have inflammation in their gut.^[34,35] Several factors can affect colonization of the gut microbiome, and the feeding type is the greatest factor on the colonization of the gut microbiome in early life. It has been suggested that eczema symptoms may be treated by changing the mix of gut bacteria or by reducing inflammation in the gut. Probiotics, which are live micro-organisms taken by mouth, such as the *Lactobacillus* bacteria found in unpasteurised milk and yoghurt, might achieve this.^[36,37] However, studies on the complex relationship between the gut microbiome and atopic dermatitis according to feeding type in early life are limited. In this study, the feeding style, the use of antibiotics, probiotics and other supplements will be investigated.

Another vital part in eczema development is the environmental issue. Environmental allergen levels are probably the most important determinant of whether or not allergy of genetically susceptible people happens. Increased exposure to sensitizing allergens and reduced stimulation of the immune system during critical periods of development are seen as the main factors driving the rising prevalence of allergic diseases. The IgE-mediated antigen presentation of allergens has been recognized as a key event in the pathogenesis of eczema.^[38,39] The main principles of eczema management are allergen and irritant avoidance, emollient use and topical or systemic anti-inflammatory treatment.^[40] The most common allergens leading to allergy in childhood are house-dust mite, grass pollen, etc. Allergens causing type-1 hypersensitivity reaction should clearly be avoided and there is some proof that reducing dust-mite exposure in the subset of susceptible patients improves eczema management.^[41] In this study, the factors such as pet at home, air quality, day care condition, family history will be examined.

Review articles suggested that prenatal maternal distress might also increase the risk of allergic disease in children.^[42,43] Recently, a birth cohort study showed that prenatal maternal psychological distress also increases the risk of eczema.^[44] This was supported by 2 more recent birth cohort studies that showed that when mothers experienced stressful life events during pregnancy, their children had an increased risk of eczema.^[45,46] Given the significant medical and psychological burden of eczema, further investigation into the association between maternal distress and subsequent eczema in offspring is warranted.

Currently, there is no cure for eczema. Proper treatment and good skin care can often control or minimize symptoms. However, prevention is the best way to manage eczema. For that reason, it is important to identify and avoid symptom triggers, such as allergens or improper diet. In long term, improving the immunity and reducing inflammation are beneficial to the affected people, especially the infants. Therefore, the aims of this study is to identify the association of early-life gut microbiome, environmental, lifestyle and maternal stress risk factors in the development of eczema. This study is significant in that it will provide evidence on the factors associated with early development of eczema. The results of this study may lead to other descriptive and interventional studies that will evaluate different approaches to prevent, treat and manage the disease of eczema.

This study started in September 2016 and is under on-going status at the present time.

Author contributions

Carmen Chan is responsible for study design.
 Stephen Tsui provide suggestions on microbiome analysis.
 TF Leung provides suggestions on recruitment of participants.
 KC Choi is responsible for data analysis.
 Judy Chan is responsible for data collection and experimental work.
 All of the authors contributed to revisions of the manuscript and its final approval.

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